Remarks

Declaration

The examiner objected to the Declaration on the basis that it failed to reference the parent U.S. application. This appears to be an error since review of the Declaration filed with the application references the parent application, U.S. Serial No. 08/268,809, and claims priority under 35 U.S.C. §120, at page two of the declaration. A copy is enclosed for the convenience of the Examiner.

Double Patenting

The double patenting issue will be addressed when the claims are otherwise allowable.

Rejection under 35 U.S.C. §112

Claims 15-17 and 42, 45-47 were rejected under 35 U.S.C. §112, first paragraph, on the basis that the application only describes addition of immobilized antibodies to immobilized antibodies. This rejection is respectfully traversed.

Page 13, at lines 1-7, describes the various types of immunoassays used to measure apolipoproteins, including radioimmunoassay, comptitive or capture systems, fluorescence immunoassay, radial immunodiffusion, nephelometry, turbidimetry, and electroimmunoassay. Page 14, lines 27-30, refer to the "preferred embodiment" being in the form of immobilized antibodies. Page 15, lines 9-17, makes reference to use of the antibodies "whether immobilized or in solution" which "can be used not only as components of dipsticks, but also in a variety of other methods, including enzyme immunoassays, radioimmunoassays as well

as fluorescent and chemiluminescent immunoassays to determine lipoproteins and apolipoproteins in biological samples". Page 15, line 25, to page 16, line 3, refers to the "second preferred embodiment" being "immunoprecipitation", which includes turbidimetry, fluroescence immunoassay, and nephelometry (all performed in solution). There is an entire section from page 40, line 19, to page 42, line 30, entitled "Use of Antibody in Solution". Reference to these sections of the application as originally filed should remove any doubt that the applicants originally intended to encompass assays using antibodies in solution as well as assays utilizing antibodies which are immobilized. It is clear from these sections, as well as others, that the inventions are the selection and combinations of the antibodies which yield the desired results; not whether they are in solution or immobilized. See also claim 12 as originally filed (directed to a turbidimetric assay, which requires that the reagents be provided in solution).

Claims 15-17, 21, 22, 28, 29, 30, 35-37, 42, and 45-47 were rejected under 35 U.S.C. §112 as lacking "essential steps".

Claims 15 and 16 have been amended to recite steps for separating out the reacted antibody-sample. It is understood that by including a separation step that this does not mean that the step requires immobilization of antibody, nor that separation requires physical separation, but can be achieved through selection of reaction conditions that causes precipitation of the complex or other methods routinely used in antibody assays. Claim 17 does not need a separation step. Claims 21, 22, 28, 29, and 30, all depend from

composition claim 18; claims 35-37 are also drawn to antibody composition claims. These do not require a separation step. Claim 42 does not require a separation step since the two measurements can be made on the same sample, for example, sequentially using a turbidimetric assay. The same comments also apply to method claims 45-47.

Claims 15-17, 21, 22, 28, 29, 30, 36, and 37 were rejected under 35 U.S.C. §112 for requiring antibodies having similar properties to specific antibodies.

The similar to language has been deleted from these claims and the antibodies defined solely by their epitope specificity, except in those cases where specific deposited antibodies are referenced. Since all antibodies described in the application are either deposited with the ATCC, or described in the public literature prior to the filing date of this application, these specificities are well understood and antibodies having the requisite specificity available to those who practice the claims.

Enclosed is a Declaration of Availability of Deposit and copies of the deposit forms with the ATCC regarding the two specifically claimed antibodies.

With regard to production of other antibodies with the claimed specificity, the application specifically exemplifies how these antibodies were produced and therefore tells one of skill in the art with great teach how to make the claimed antibodies. There is no requirement for the protein sequence of the epitope, as demonstrated by the examples. See in particular Example 2, pages 47- 51 (production of antibody to delipidated, reduced, carboxymethylated Apo B-100), and example 12, pages 70-74, production of recombinant

antibody. The licensee of this application, Sigma, St. Louis, MO, has made other antibodies of similar reactivity, which are now in assays being developed and tested for the clinical lab market.

The examples describe how to perform each step of the process that can be used to obtain antibodies as claimed that "specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of a specific lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein". Moreover, Applicants have demonstrated specific examples of such antibodies: Antibodies were made which are immunoreactive with Apo A-I (to two different epitopes, in Examples 1 and 8); Apo B-100 (Example 2); Apo A-I and Apo A-II (Example 10); Apo C-III (Example 11); and Apo E (example 11). An antibody which is not specific ("Pan B (D6) antibody) is described in Example 7, which is useful as the second antibody in the sandwich assay of claims 12, 13, 39, and 43-45.

A person of ordinary skill in the art, upon reading the specification, would understand how to make and use the claimed methods and reagents for use therein. Merely because screening is required does not mean the methods are unpredictable and non-reproducible. The key was determining that an antibody that is immunoreactive with a stable, conformation independent epitope which is not influenced by lipid content was needed and then designing a process which would yield such an antibody, not the routine screening which follows.

Claims 15-17, 18, 23, 25, 27, 28, 29, 30, 35, 42, and 45-47 were rejected under 35 U.S.C. §112 as not enabled for "polyclonal antibodies". This rejection does not make sense

as to all of the claimed antibodies; only to those where specificity to a specific epitope is required. In fact, in the case of Pan B antibodies, polyclonal antibodies or mixtures of monoclonal antibodies are essential. Those claims where specificity is required have been amended to recite that the antibodies are "monoclonal" antibodies, or already recite that the antibodies are monoclonal or recombinant antibodies, or "fragments thereof".

Claims 28, 29 and 30 have been amended to recite "further comprising".

The dependency of claim 41 has been corrected.

Rejections under 35 U.S.C. §102 or §103

Claim 17 was rejected under 35 U.S.C. §102(b) as disclosed by either Koren, et al., Atherosclerosis 95, 157-170 (1992) or Koren, et al., Clin. Chem. 33(1), 38-43 (1987). This rejection is respectfully traversed.

The claim requires that the antibody to Apo AI be reacted with both LPA-1 and LPA-1:A-II lipoprotein particles, the amount of Apo A-I associated with both types of particles calculated, then the amount of Apo A-II associated with the LPA-I:A-II determined. These steps are not shown by either Koren.

Claims 18, 20, 23, 25, 27 and 30 were rejected under 35 U.S.C. §102(a) as disclosed by WO 93/18067 by Abbott (Kundu) or under §102(b) as disclosed by La Belle, et al., Clinica Chimica Acta 191, 153-160 (1990). These rejections are based solely on the showing by Kundu (or La Belle) of an antibody which binds Apo B without analyzing the inclusion in each one of these claims additional steps or reagents which are not shown by Kundu or La

Belle. The rejection on this basis under §102 is therefore improper. However, it is also improper because Kundu (or La Belle - they are the same antibodies) does not disclose a monoclonal antibody which is reactive with a conformationally-independent antibody.

Anticipation requires the disclosure, in a single prior art reference, of every element of the claim. *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81, 90 (Fed. Cir. 1986). Absence of a claimed element from a prior art reference negates anticipation. *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 224 U.S.P.Q. 409 (Fed. Cir. 1984).

A rejection under 35 USC §102/103(a) based on inherency is improper unless the office action sets forth a *prima facie* basis establishing the asserted inherency. As noted in the Manual of Patent Examining Procedure (MPEP), the EXAMINER MUST PROVIDE RATIONALE OR EVIDENCE TENDING TO SHOW INHERENCY. See MPEP §2112 Rev. 3, July 1997 (capitalization in original). In fact, for claims directed to processes in which a required element of the claim is asserted as being inherent in a prior art process, the Examiner must demonstrate that "the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art." *In re Best*, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) (emphasis in original); *Continental Can Company USA, Inc. v. Monsanto Co.*, 20 USPQ2d 1746 (Fed. Cir. 1991); *Ex parte Tanksley*, 37 USPQ2d 1382, 1385 (Bd. Pat. App. & Inter. 1994); *See also Hansgirg v. Kemmer*, 40 USPQ 665, 667 (CCPA 1939) (stating that "[i]nherency may not be established by probabilities or possibilities," but rather, must be

based on a "natural result flowing from the operation as taught.") The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic. *Electro Medical Systems, S.A. v. Cooper Life Sciences, Inc.*, 34 F.3d 1048, 32 USPQ2d 1017 (Fed. Cir. 1994).

Kundu describes antibodies that bind to the apo T2 fragment of LDL and have "low (less than 20%)" cross reactivity with VLDL, IDL, and Lp(a), such as 4B5.6, which was obtained from Alexander Karu (Kundu, page 23, lines 31-34). Kundu describes the importance of selection of an appropriate lipoprotein specific binding agent on page 20, lines 8-25. The criteria are specificity to a particular lipoprotein and compatibility with the cholesterol binding antibody. Kundu does not disclose that it is important for 4B5.6 or any of the antibodies taught therein to specifically bind to a stable, conformation independent epitope which is not influenced by lipid content. In fact, in Figure 7A Kundu specifically teaches that antibody 4B5 (sic) is **conformation dependent**. See also the discussion at page 33, lines 4-29. Therefore the antibody of Kundu is not the same as, and does not anticipate, the claimed antibodies.

Nor does Kundu teach that any of the antibodies disclosed therein will specifically bind to a stable, conformation independent epitope which is not influenced by lipid content. The Examiner has no support for her assertion that the antibodies taught by Kundu inherently possess the claimed property. T2 is a large fragment of Apo-B, including 1,278 amino acids, an approximate molecular weight of 130,000, and numerous epitopes against which

antibodies could be generated. The argument that because 4B5.6 binds to the same very large region (T2) of Apo B it "would reasonably be expected to have the same properties as the Apo B monoclonal antibody disclosed by [Applicants]" is insupportable when one notes the size of the T2 region. Kundu does not disclose the importance of the specifically recited properties of the claimed antibodies nor how to produce such antibodies.

La Belle (note that this publication is also authored by Alexander Karu) discusses the same antibody as Kundu, the LDL specific binding antibody 4B5.6. See La Belle at page 155 and at page 159: "Since no detectable alterations were found in the protein component of LDLs showing the rise in rate, nor did any of the modifications we made to Apo B produce any increase in the ELISA rates, it is possible that the rate changes reported here were due to changes in the lipid component of LDL which in turn affected interaction of the Apo B with the antibodies." Although La Belle screened for this antibody by electrophoresis using denatured, delipidated Apo B-100, **the antibody was not generated against denatured, delipidated Apo B-100**. Therefore, it cannot be assumed to have the same properties. Moreover, simply because an antibody binds to a delipidated apolipoprotein is not evidence that the binding is independent of conformation or lipid content. In fact, an antibody that is immunoreactive with delipidated apolipoprotein may bind specifically to an epitope which is typically shielded by lipid, in which case immunoreactivity to the native, lipidated lipoprotein would more than likely be very much affected by lipid content. The key to Applicants' claimed methods is that the antibodies are generated to denatured, delipidated antigens. The

screening method used by Applicants to determine lipoprotein specificity was ELISA assays, employing **native** lipoproteins (as would be present in clinical specimens) (see page 48, lines 7-20), not gel electrophoresis using denatured apolipoproteins (requiring extensive laboratory preparations precluding use in a quick inexpensive assay).

The Examiner makes the same argument as to La Belle that, since the antibody taught by La Belle binds to the T2 region, it "would reasonably be expected to have the same properties as the Apo B monoclonal antibody disclosed by [Applicants]". This argument rings false when one considers the size and numerous epitopes that have been identified in the T2 region of LDL. It is just as likely, or even more so, that the prior art antibody binds to a conformational or lipid dependent different epitope on T2, particularly since there is no indication that it was generated against denatured, delipidated antigen, and it was tested only against denatured antigens, which cannot be used to exclude antibodies that bind epitopes differently in their native state or in the presence of different amounts of lipid.

It was emphasized in the Office Actions that 4B5 "DID NOT show an increase in the rate" of binding to stored LDL and is "deemed to bind to a conformational independent epitope". While it is true that 4B5 does not show an increase in rate of binding, La Belle's results clearly demonstrate a significant <u>decrease</u> in the rate of binding for 4B5. See La Belle at page 157 Figs 2, A, B, and C. It is generally known that conformational changes could cause a <u>decrease</u> as well as an increase in antibody binding. Therefore, La Belle does not teach that 4B5 binds to a conformation independent epitope.

Claims 18, 20, 23, 25, 27, 28, 29, 30 and 35 were also rejected under 35 U.S.C. §103 as obvious over Kundu or La Belle, et al., in combination with Koren, et al. Atherosclerosis 95, 157-170 (1992). These rejections are also respectfully traversed.

The examiner has rejected the claims on the basis that the recited reagents must be disclosed somewhere in this collection of art. This is not a proper analysis under §103, however, which expressly requires not only a showing of each element but the motivation to combine the elements as applicants have done with a reasonable expectation of success. Applicants have done several things not described in the prior art: prepare conformationally independent antibodies to apolipoproteins, combine different reagents to define the relative ratios of different apolipoproteins, and more importantly, the relative ratios of different types of lipoproteins (HDL, VLDL, and LDL). Some of the claimed assays require distinct steps that measure three and four variables; none of the prior art ever recognized this. The examiner's analysis reflects a cursory review; not a well founded rejection. Accordingly, to facilitate prosecution, should the claims again be rejected, the applicants and undersigned request an interview to discuss the prior art, the claimed methods and reagents, and why these are patentably distinct.

Allowance of all claims 15-18, 20-23, 25, 27-37, 41, 42, and 45-47, as amended, is earnestly solicited. All claims as pending upon entry of this amendment are attached in an appendix for the convenience of the examiner.

Respectfully submitted,

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Date: July 21, 1998

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CERTIFICATE OF MAILING UNDER 37 CFR § 1.8(a)

I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Patrea L. Pabst

Date: July 21, 1998

Appendix: Claims as Amended

15. (twice amended) A method for determining the relative ratio of VLDL to HDL in a biological sample comprising

determining the amount of Apo C-III present in the VLDL in the sample by

providing Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing monoclonal antibody immunoreactive with Apo C-III [having binding affinity and specificity similar to XbA_3],

contacting the antibody reactive with Apo C-III with the biological sample to form complexes between the antibody and the Apo C-III containing lipoprotein particles,

contacting the Pan B antibody with the biological sample,

separating the complexed antibody-lipoprotein particles from the biological sample, and

determining the amount of Apo C-III associated with Apo B, which is the amount of Apo C-III present in VLDL in the sample; and

determining the amount of Apo C-III present in the HDL in the sample by

providing Apo A-I monoclonal antibody immunoreactive specifically with Apo A-I [having a binding affinity and specificity similar to $AIbD_5$ and $AIbE_2$],

providing monoclonal antibody immunoreactive with Apo C-III [having binding affinity and specificity similar to XbA_3],

contacting the antibody reactive with Apo C-III with the biological sample to form complexes between the antibody and the Apo C-III containing lipoprotein particles,

contacting the anti-Apo A-I antibody with the biological sample,

separating the complexed antibody-lipoprotein particles from the biological sample,

determining the amount of Apo C-III associated with Apo A-I, which is the amount of Apo C-III present in HDL in the sample, and

determining the ratio of Apo C-III present in VLDL in the sample and Apo C-III present in HDL in the sample which is the ratio of VLDL to HDL.

16. (twice amended) A method for determining the relative ratio of VLDL to HDL comprising

determining the amount of Apo E present in the VLDL in the sample by

providing Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing monoclonal antibody [immunoreactive with Apo E having binding affinity and specificity similar to EfB_1] which binds to Apo E associated predominantly with VLDL,

contacting the antibodies reactive with Apo E associated with VLDL with the biological sample to form complexes between the antibodies and Apo E containing particles,

separating the complexed antibody-ApoE containing particles from the biological sample,

contacting Pan B antibody with the biological sample, and

determining the amount of Apo E associated with Apo B which is the Apo E present predominantly in VLDL in the sample;
and

determining the amount of Apo E present in the HDL in the sample by

providing Apo A-I monoclonal antibody immunoreactive specifically with Apo A-I [having a binding affinity and specificity similar to $AIbD_5$],

providing monoclonal antibody [immunoreactive with Apo E having binding affinity and specificity similar to EfD_3,] which binds to Apo E predominantly associated with HDL,

contacting the antibodies reactive with Apo E to the biological sample to form complexes between the antibodies and Apo E containing particles,

separating the complexed antibody-ApoE containing particles from the biological sample,

contacting Pan B antibody with the biological sample,

determining the amount of Apo E associated with Apo A-I, which is the amount of Apo E present in HDL in the sample, and

determining the ratio of Apo E present in VLDL in the sample and Apo E present in HDL in the sample which is the ratio of VLDL to HDL.

17. (twice amended) A method for determining the relative ratio of LPA-I and LPA-I:A-II lipoprotein particles in a biological sample comprising

providing anti-Apo A-I monoclonal antibody immunoreactive specifically with Apo A-I [having a binding affinity and specificity similar to $AIbD_5$,],

providing anti-Apo A-II monoclonal antibody immunoreactive specifically with Apo A-II [having a binding affinity and specificity similar to CdB_5];

contacting the anti-Apo A-I antibody [having a binding affinity and specificity similar to $A1bE_2$] with the sample to form complexes with both LPA-I and LPA-I:A-II

and determining the quantity of Apo A-I associated with both LPA-I and LPA-I:A-II lipoprotein particles; and

contacting the anti-Apo A-II antibody with the biological sample to form complexes with LPA-I:A-II and determining the quantity of Apo A-II associated with the LPA-I:A-II.

18. (twice amended) A composition for determining the concentration of a lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein in a biological sample comprising:

antibody molecules specifically immunoreactive with a specific lipoprotein or apolipoprotein, wherein the antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.

20. (amended) The composition of claim 18 wherein the antibodies are monoclonal antibodies.

21. The composition of claim 18 wherein the antibody is the anti-LDL monoclonal antibody produced by the hybridoma cell line HB_3cB_3 ATCC designation number HB 11612.

22. The composition of claim 18 wherein the antibody is a recombinant anti-LDL $RcB_3M_1D_4$ ATCC designation number 69602.

23. (twice amended) The composition of claim 18 further comprising a second monoclonal antibody immunoreactive with a second distinct epitope of the lipoprotein or apolipoprotein which is immunoreactive with the first antibody.

25. (amended) The composition of claim further comprising at least one internal standard comprising a known amount of a particular lipoprotein, lipoprotein lipid, or apolipoprotein.

27. The composition of claim 18 wherein the apolipoprotein is selected from the group consisting of Apo A-I, Apo A-II, Apo B, Apo C-III, and Apo E.

28. (twice amended) The composition of claim 18 for determining the relative ratio of VLDL to HDL further comprising

Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

monoclonal antibody specifically immunoreactive with Apo C-III [having binding affinity and specificity similar to XbA_3], and

monoclonal Apo A-I antibody immunoreactive specifically with Apo A-I [having a binding affinity and specificity similar to $AIbD_5$ and $AIbE_2$].

29. (twice amended) The composition of claim 18 for determining the relative ratio of VLDL to HDL further comprising

Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

monoclonal antibody [immunoreactive with Apo E having binding affinity and specificity similar to EfB_1] which predominantly binds to Apo E associated with VLDL ,

monoclonal Apo A-I antibody immunoreactive specifically with Apo A-I [having a binding affinity and specificity similar to $AIbD_5$], and

monoclonal antibody [immunoreactive with Apo E having binding affinity and specificity similar to EfD_3] which predominantly binds to Apo E in HDL.

30. (twice amended) The composition of claim 18 for determining the relative ratio of LPA-I and LPA-II lipoprotein particles comprising

monoclonal Apo-A-I antibody which binds Apo A-I lipoproteins in human plasma [having a binding affinity and specificity with Apo $AIbD_5$]; and

monoclonal Apo A-II antibody immunoreactive specifically with Apo A-II [having a binding affinity and specificity similar to CdB_5].

35. An antibody molecule specifically immunoreactive with LDL that does not significantly cross-react with other lipoproteins in whole blood, blood plasma or blood serum, wherein the molecule is selected from the group consisting of monoclonal antibodies,

recombinant antibodies, and fragments thereof and wherein the antibody specifically binds to a stable, conformation independent epitope which is uninfluenced by the lipid content.

36. The antibody molecule of claim 35 wherein the antibody is the anti-LDL monoclonal antibody produced by the hybridoma cell line HB_3cB_3 ATCC designation number HB 11612.

37. The antibody molecule of claim 35 wherein the antibody is a recombinant anti-LDL $RcB_3M_1D_4$ ATCC designation number 69602.

42. (amended) A method for determining the relative ratio of LDL to HDL in a biological sample comprising

adding to the sample monoclonal antibody molecules immunoreactive with low density lipoprotein and not cross-reactive with high density lipoprotein and determining the amount of low density lipoprotein;

adding to the sample monoclonal antibody molecules immunoreactive with high density lipoprotein and not cross-reactive with low density lipoprotein and determining the amount of high density lipoprotein; and

determining the ratio of the amount of low density lipoprotein with the amount of high density lipoprotein.

45. (amended) A method for determining the relative ratio of first and second lipoproteins in a biological sample, comprising:

determining the amount of first lipoprotein in the sample by

contacting a first monoclonal antibody immunoreactive with a first apolipoprotein on the first lipoprotein with the sample to form complexes between the first antibody and the first apolipoprotein,

contacting a second monoclonal antibody immunoreactive with a second apolipoprotein on the first lipoprotein with the sample to form complexes between the second antibody and the first antibody:first lipoprotein complexes,

determining the amount of second apolipoprotein associated with the first apolipoprotein, which is the amount of second apolipoprotein associated with the first lipoprotein;

determining the amount of second lipoprotein in the sample by

contacting a third monoclonal antibody immunoreactive with a third apolipoprotein on the second lipoprotein with the sample to form complexes between the third antibody and the third apolipoprotein,

contacting a fourth monoclonal antibody immunoreactive with a fourth apolipoprotein on the second lipoprotein with the sample to form complexes between the fourth antibody and the fourth antibody:second lipoprotein complexes,

determining the amount of fourth apolipoprotein associated with the third apolipoprotein, which is the amount of fourth apolipoprotein associated with the second lipoprotein; and

determining the ratio of first and third apolipoproteins which is the ratio of first and second lipoproteins.

46. (amended) The method of claim [43] 45, wherein the first apolipoprotein is the same as the third apolipoprotein and at least one of the second or fourth apolipoprotein is specific for the first or second lipoprotein, respectively.

47. (amended) The method of claim [43] 45, wherein the first antibody is the same as the third antibody.